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RESEARCH ARTICLE

EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *PROTEUS SPECIES* ISOLATED FROM CLINICAL SPECIMENS FROM SELECTED HOSPITALS IN JIGAWA STATE, NORTH-WEST NIGERIA.

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Abstract: *Proteus* species are found in multiple environmental habitats, including long-term care facilities, hospitals, and can also cause both community and nosocomial infections. For a long time, *Proteus* was known to be susceptible to beta-lactam antibiotics but nowadays they become resistant. Hence, this study aims to determine the prevalence, antibiotic susceptibility pattern of extended spectrum beta lactamase (ESBL) producing *Proteus species* across Jigawa State, Northwest Nigeria. 1854 different clinical specimens were analysed of which 191 *Proteus species* were isolated through standard biochemical tests and used for this study from selected hospital between November, 2021 to August, 2022. Modified Kirby-Bauer disk diffusion method was used to test the susceptibility of the *Proteus* isolates to nine different antimicrobial agents. Double Disc Synergy Test (DDST) was used for phenotypic detection of ESBL in isolates. The prevalence of ESBL producing *Proteus mirabilis* was 11.8%. None of the *Proteus vulgaris* isolates in this study was found to be ESBL producers. Male patients were infected with more ESBL producers than those from female counterparts (Male vs Female; 12.5% vs 4.8%). *Proteus mirabilis* were observed to be significantly more susceptible to Ampicillin (P= 0.003), Gentamycin (P=0.024) and Cotrimoxazole (P=0.014). All ESBL producing *Proteus mirabilis* were resistant to three or more classes of antibiotics used in this study. This study reveals the occurrence of ESBL producing *Proteus species* in this environment. All the ESBL producing *Proteus mirabilis* encountered in this study exhibited multidrug resistance. Prudent use of antimicrobial agents is advocated in order to tame the trend.

KEYWORDS: *Proteus species*, Prevalence, ESBL producing *Proteus mirabilis*, Multidrug resistance

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INTRODUCTION:

The phenomenal evolution and increase of multidrug-resistance of many bacterial pathogens is increasing and representing a growing public health problem in the world. Multidrug-resistance of *Proteus species* calls for regular review of antimicrobial sensitivity pattern among clinically isolated *Proteus species* in order to be able to decide which antibiotic to be prescribed [1]. The routine use of antibiotics in both medical and veterinary medicine has resulted in wide spread antibiotic resistance and development of antibiotic resistance genes especially within the gram-negative organisms [2]. Plasmids with Multidrug-resistant genes are common among the family of *Enterobacteriaceae*. Historically, *Proteus* species were known to be free of the beta-lactamase genes However, *Proteus*, as a member of the family *Enterobacteriaceae*, can acquire the plasmids from other members of the family [3, 4].

Members of the family Enterobacteriaceae commonly express plasmid-encoded β-lactamases, extended spectrum beta lactamase (ESBL) (e.g., TEM-1, TEM-2, and SHV-1) which confer resistance to penicillin but not to expanded-spectrum cephalosporins. Beta-lactamase are enzymes produced by some bacteria and are responsible for their resistance to certain groups of antibiotics, like penicillin's, cephalosporins and carbapenem [5] β-lactamase deactivates β-lactam antibiotics, by breaking and opening their common molecular structure (β- lactam ring). Some of these enzymes include extended spectrum β-lactamase (ESBL), and carbapenems. The first β-lactamase was detected during the 1960s [6]. Extended spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae,* are among the most problematic multidrug resistance (MDR) bacteria worldwide [7].

ESBLs are the beta-lactamases capable of hydrolyzing penicillin, broad-spectrum cephalosporins, and monobactams, and are generally derived from TEM and SHV-type enzymes but, may not affect cephamycin's and carbapenems. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species [8].

Gram negative *Enterobacteriaceae* expressing AmpC and Extended-Spectrum-Beta-Lactamases are among the most multi-drug-resistant pathogens in hospitals and they are spreading worldwide [9]. Reports on the prevalence of ESBL-producing *Proteus species* are few in Jigawa State, Nigeria. This study was undertaken to characterize the *Proteus species* isolated from clinical samples, antibiotics sensitivity pattern and the ESBLs produced by clinical isolates from selected hospitals in Jigawa State, Nigeria.

MATERIALS AND METHODS:

Study Area

The study was carried out on all *Proteus* organisms isolated from clinical samples (wound swabs, urine, ear swabs, high vaginal swab/endo-cervical swab, sputum, aspirates) from selected hospitals in Jigawa State. The hospitals include Hadeija General Hospital, Dutse General Hospital, Rasheed Shekoni Specialist Hospital Dutse (RSSH) and Federal Medical Centre, Birnin-kudu between November, 2021 to August, 2022 and sample size of 191 samples was used based on the reports of 14.6% prevalence rates of *Proteus* infections in Kano [10]. the sample size was determined using the formula described by Naing [11].

Cultivation and Identification

The clinical samples were aseptically collected and inoculated on plates of Blood agar, and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37^0C for 24 hrs. Suspected *Proteus* colonies were identified through biochemical tests according to [12]. Based on whether they were positive for Indole production; H2S gas production; Citrate utilization and urease reactions; and negative for lactose fermentation. Indole production differentiates *P. vulgaris* isolates from *P. mirabilis*.

Antimicrobial Susceptibility Test

Modified Kirby-Bauer disk diffusion method Cheesbrough, [13] was used to test the susceptibility of

the *Proteus* isolates to different antimicrobial agents (obtained from Mast Diagnostics, UK): Amoxicillin (30 µg), ampclox (10 µg), augmentin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), co-trimoxazole (25 µg) and imipenem (10 µg). By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs was applied to the surface of the inoculated agar and the plates incubated overnight at 37 ºC. The diameter of zone of growth-inhibition was observed, measured and recorded sensitive (S) or resistance (R) according to CLSI, [14].

Screening for Suspected ESBL Producers

ESBL producers were screened by disk-diffusion method using ceftazidime, cefotaxime and ceftriaxone. If the isolates are resistant to any of these drugs, they are considered as suspected ESBL producers [15].

Detection of ESBL Producers by Double-Disk Synergy (DDS) Test

ESBL producers were further confirmed for ESBL production by DDS test as described by Driuex [16]. Amoxiclav disk was placed at the center of the inoculated Mueller-Hinton agar plate. Third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) were placed 15 mm apart from center of the amoxiclav disk. After incubation at 37^0C for 24 hours, a clear extension of the edge of the inhibition zone of cephalosporins disks towards amoxiclav disk was interpreted as ESBL producer. The expansion occurred because of clavulanic acid present in augmentin disc inhibits ESBL enzyme produced by the organism. Non–ESBL-producing organism (Escherichia coli ATCC 25922) and an ESBL-producing organism (*Klebsiella pneumoniae* ATCC 700603) were used as quality controls.

Ethical Clearance

Ethical approval was obtained from the ethical committee of Federal Medical Centre, Birnin Kudu Hospital management and Jigawa State Ministry of Health, Dutse.

Data Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS® 20, USA). Descriptive statistics was used to describe the relevant variables and comparisons performed using chi-square test.

RESULTS

Prevalence and Distribution of ESBL Producing *Proteus species*

The prevalence of ESBL producing *Proteus mirabilis* was 11.8%. None of the *Proteus vulgaris* isolates in this study was found to be ESBL producers. Indeed, *Proteus mirabilis* isolates were over eleven times significantly more likely (OR=11.164, P=0.018) to produce ESBL than *Proteus vulgaris* isolates. No ESBL producing bacteria was isolated from urine and High vaginal swabs. However, in this study, ear swab accounted for the most number 6 (18.7%) of ESBL producing bacteria, while sputum accounted for the least 1(9.0%). Furthermore, highest ESBL producing *Proteus spp* was recorded in FMC Kudu 13.0%, while the least (6.8%) was observed among isolates in General Hospital Hadejia. (Tables 1, 2 and 3).

Table 1: Prevalence of ESBL Among *Proteus species* in the Study

Bacteria	N	No ESBL Pos (%)	OR	95% CI	P value
Proteus mirabilis	153	18 (11.8)	11.164	0.658, 189.3	0.018*
Proteus vulgaris	38	0 (0)			

N- number of isolates screened; **ESBL**-extended spectrum beta-lactamase; **OR**-odd ratio; **CI**-confidence interval; -

Table 2: Prevalence of ESBL with Respect to Source of Bacteria Isolation

Clinical specimen	N	No ESBL Pos (%)	P value
Wound swab	107	11 (10.3)	0.134
Urine	27	0 (0.0)	
Ear swab	32	6 (18.7)	
High Vaginal swab	5	0 (0,0)	
Sputum	20	1(9.0)	

N- number of isolates; **ESBL**- extended spectrum beta-lactamase

Table 3: Prevalence of ESBL Producing *Proteus mirabilis* with Respect to Location of Subjects

Clinical specimen	N	No ESBL Pos (%)	P value
FMC Kudu	69	8(11.6)	0.728
General Hospital, Dutse	44	4 (9.1)	
General Hospital, Hadeija	44	3 (6.8)	
RSSH, Dutse	34	3 (8.8)	

N- number of isolates; **ESBL**- extended spectrum beta-lactamase

Age and Sex Distribution of ESBL Producing *Proteus mirabilis*

With respect to gender, isolates recovered from male subjects were found to be more ESBL producers than those from female counterparts (Male vs Female; 12.5% vs 4.8%). Although, *Proteus mirabilis* from male subjects were found to be about three times (OR=2.795) more likely to be ESBL positive than those from females, statistics did not show any significant different in rate of ESBL production with respect to gender. (P=0.125). The prevalence (18.9%) of ESBL producing *Proteus mirabilis* was highest among participants within the age group of 22-31 years. None of the isolates recovered from participants aged 62 years and above was found to be an ESBL producer. (Table 4 and 5).

Table 4: Prevalence of ESBL Producing *Proteus mirabilis* with Respect to Gender of Subjects

Gender	N	No ESBL pos (%)	OR	95% CI	P value
Male	129	15 (11.6)	2.795	0.779, 9.945	0.125
Female	62	3 (4.8)			

N- number of isolates screened; **ESBL**-extended spectrum beta-lactamase; **OR**-odd ratio; **CI**-confidence interval

Table 5: Prevalence of ESBL Producing *Proteus mirabilis* With Respect to Age of Subjects

Age (years)	N	No. ESBL Pos (%)	P value
2- 11	39	5 (15.4)	0.242
12-21	30	2 (6.7)	
22-31	37	7 (18.9)	
32-41	38	2 (5.3)	
42-51	23	1 (4.3)	
52-61	14	1 (7.4)	
62-71	10	0 (0.0)	

N- number of subjects; **ESBL**- extended spectrum beta-lactamase

Prevalence of MDR *Proteus species* and Association with ESBL Production

A total of 27 (17.6%) *Proteus mirabilis* isolates were found to be resistant to three or more classes of antibiotics used in this study. This was higher than 5.3% observed among *Proteus vulgaris* isolates. Compared to *Proteus vulgaris*, *Proteus mirabilis* were about four times (OR=3.857) more likely to be multi-drug resistant, albeit the difference was not statistically significant (P = 0.075). All ESBL producing *Proteus species* were resistant to three or more classes of antibiotics used in this study. However, a total of 10(5.8%) of non-ESBL *proteus* isolates were also found to be multi-drug resistant. ESBL producing *Proteus spp* were found to have over six hundred times significantly (0R=603.57; P<

0.0001) more likely to be multidrug resistant than those negative for ESBL. (Table 6 and 7).

Table 6: Prevalence of MDR Bacteria

BACTERIA	N	MDR Pos (%)	OR	95%CI	P value
Proteus mirabilis	153	27 (17.6)	3.857	0.875, 17.007	0.075
Proteus vulgaris	38	2 (5.3)			

N- number of isolates; **MDR**- multidrug resistant; **OR**-odd ratio; **CI**- confidence interval

Table 7: Association Between ESBL Production and Multidrug Resistant Bacteria

VARIABLE	N	MDR Pos (%)	OR	95% CI	P value
ESBL Positive	18	18 (100.0)	603.57	34.005, 10713.1	< 0.0001*
ESBL Negative	172	10 (5.8)			

N- number of isolates; **MDR**- multidrug resistant; **ESBL**- extended spectrum beta-lactamase; **OR**-odd ratio; **CI**- confidence interval; *-statistically significant

Plate 1: ESBL Producing *Proteus species* Detected by DDST Method (Field Work, 2022).

DISCUSSION:

The presence of ESBL producing bacteria is striking rapidly worldwide, hence the increase of resistance to antibiotics and the emergence of multidrug-resistant ESBL producers are becoming a public health problem, causing clinical failure of empirical antibiotic treatment [17].

Based on the report of meta-analysis of Systematic review on the prevalence of extended-spectrum beta lactamase-producing Gram-negative bacteria in Nigeria, there is scanty report from the Northwest region and no information at all from Jigawa State concerning ESBL producing *Proteus species* and phenotypic detection of ESBL [18].

Of the one hundred and ninety-one (191) *Proteus* isolates analyzed for ESBL production with the double disk synergy test, (9.4%) turned out positive. This percentage is lower compared to reports from other parts of the country and elsewhere in the world [19-21].

The prevalence of *Proteus mirabilis* ESBL producing isolates was 11.8%. This finding was in contrast with report of 52% in *Proteus mirabilis* in Italy [22]. However, *Proteus species* have been reported by some workers to be infrequent producer of ESBL [23]. Furthermore, isolates recovered from male subjects were found to be more ESBL producers than those from female counterparts (Male vs Female; 11.6% vs 4.8%). It was hypothesized that males in general have more obstructive uropathies leading to frequent urinary catheterization and have more chronic pulmonary and cardiovascular diseases that in turn lead to increased hospitalization and higher prevalence [21].

Among the clinical specimens, Wound swabs had the greatest number of *Proteus species* while high vagina swab had the least. Federal Medical Centre (FMC) Birnin- kudu had the highest proportion of *Proteus species,* while the least was found in Rasheed Shekoni Specialist Hospital (RSSH) Dutse. This observation is in contrast with other studies who found that ESBL prevalence in *Proteus species* was highest for blood (61.2%) and urine (16.41%) specimens and (22.2%)

ESBL in blood samples followed by urine (17.6%) [17, 24].

The prevalence (18.9%) of ESBL producing *Proteus* spp was highest among participants within the age group of 22-31 years. None of the isolates recovered from participants aged 62 years and above was found to be an ESBL producer. However, age was not identified as a risk factor for harboring ESBL producing *Proteus spp* in this study (P=0.242). This was in line with the report where ESBL producing *Proteus species* were isolated in all age groups except aged 60 years and above [25].

All the ESBL encountered exhibited multi drug resistant. This resistance observed in the present study could be due to the wide spread abuse and misuse of these drugs owning to its availability and affordability. The observed reduced susceptibility of the cephalosporin in this study is a strong indication to the presence of multi drug resistant *Proteus species* and a possible outbreak of resistant nosocomial *Proteus species* infection which calls for increased surveillance and judicious antibiotic use to curtail the trend. These studies establish that there is strong correlation between antibiotic resistance and ESBL production in *Proteus species* because all the isolates encountered exhibited multi drug resistant.

CONCLUSION:

All the ESBL producing *Proteus mirabilis* encountered in this study exhibited multidrug resistance. Wound swab and urine samples had a higher prevalence of ESBL producers. Prudent use of antimicrobial agents is advocated in order to tame the trend. Finally, this research work had provided the needed information to establish the national data on the trend of ESBL production in enterobacteriaceae in Nigeria.

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